**Supplementary information 1:**

A screening bio-analytical method for *Enterococcus faecalis* RNPP-type quorum sensing peptides in murine faeces.

**Supplementary information Table 1: Characteristics of murine faeces samples.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample ID** | **Sampling location** | **Strain** | **Sampling date** | **Remarks** |
| FM1 | Janssen Pharmaceutica B023, Beerse, Belgium | GRM2 | 16/08/2017 | - |
| FM2 | TauP3012 |
| FM3 | PDAPP |
| FM4 | PS19 |
| FM5 | BACE1 |
| FM6 | Janssen Pharmaceutica 020FB, Beerse, Belgium | SCID Beige |
| FM7 | NMR1-Nude |
| FM8 | C75BC/6J/ABCM/N/J |
| FM9 | Janssen Pharmaceutica 020SB, Beerse, Belgium | C3H/J |
| FM10 | BI/6 |
| FM11 | NMRI |
| FM12 | UZ Ghent animal facility, Ghent, Belgium | Hepatology breeding mice cage 1; strain not specified | August 2017 | Mice received treatment via medicinated feed with 150 ppm fenbenzadole |
| FM13 | Hepatology breeding mice; strain not specified | 21/04/2017 | Mix of 4 cages |
| FM14 | DruQuaR animal facility, Merelbeke, Belgium | ICR CD1 | 07/06/2016 | Transport boxes |
| FM15 | 12/06/2016 | Fresh faeces from cages (< 1h) |
| FM16 | UZ Ghent animal facility, Ghent, Belgium | Hepatology breeding mice cage 2; strain not specified | August 2017 | Mice received treatment via medicinated feed with 150 ppm fenbenzadole |
| FM17 | Hepatology breeding mice cage 3; strain not specified |
| FM18 | Hepatology breeding mice cage 4; strain not specified |
| FM19 | Hepatology breeding mice cage 5; strain not specified |
| FM20 | Hepatology breeding mice cage 6; strain not specified |

**Supplementary information Table 2: Mass spectrometric settings.**

|  |
| --- |
| **Mass spectrometric settings** |
| **Source offset** | 50.0 V |
| **Capillary voltage**  | 3.00 kV |
| **Cone voltage**  | 30.00 V |
| **Source temperature** | 150°C |
| **Desolvation gas flow** | 1000 l/h |
| **Desolvation temperature** | 500°C |
| **Collision energy MS1** | 4 eV |
| **Cone gas flow** | 150 l/h |
| **Collision gas flow** | 0.16 ml/min |
| **Nebuliser gas flow** | 7.00 bar |
| **Probe position**Vernier probe adjuster:Vertical probe adjuster: | 5.200.7 mm |
| **LM resolution 1:** | 2.5 |
| **HM resolution 1:** | 14.9 |
| **Ion energy:** | 0.1 |
| **LM resolution 2:** | 2.8 |
| **HM resolution 2:** | 14.9 |
| **Ion energy:** | 0.8 |
| **Divert valve** | 0.00 min - 2.00 min Waste2.01 min -9.30 min LC9.31 min - 15.00 min Waste |

**Supplementary information Table 3: Multiple Reaction Monitoring settings of the 9 RNPP peptides.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trivial name** | **Amino acid sequence** | **Multiple Reaction Monitoring** | **Collision energy (eV)** | **Time frame (min)** | **Run** |
| **Quantifier****(precursor ion > fragment ion)** | **Qualifier****(precursor ion > fragment ion)** |
| cAM373 | AIFILAS | 734.37 > 332.10 | 734.37 > 445.11 | 32 | 0-7.5 | Run 1 |
| iCF10 | AITLIFI | 790.37 > 512.16 | 790.37 > 659.18 | 20 | 7.3-15 | Run 1 |
| iPD1 | ALILTLVS | 829.43 > 625.21 | 829.43 > 724.24 | 21 | 6.6-7.8 | Run 1 |
| cPD1 | FLVMFLSG | 913.38 > 751.14 | 913.38 > 638.07 | 25 | 6.9-15 | Run 2 |
| cAD1 | LFSLVLAG | 819.46 > 560.13 | 819.46 > 744.38 | 25 | 6.6-7.8 | Run 1 |
| iAD1 | LFVVTLVG | 847.44 > 772.23 | 847.44 > 673.19 | 22 | 6.7-7.8 | Run 2 |
| cCF10 | LVTLVFV | 790.42 > 314.10 | 790.42 > 409.11 | 33 | 6.9-8.1 | Run 1 |
| iAM373 | SIFTLVA | 750.28 > 431.06 | 750.28 > 544.06 | 30 | 6.5-7.8 | Run 2 |
| cOB1 | VAVLVLGA | 741.40 > 383.12 | 741.40 > 482.16 | 30 | 0-7.3 | Run 2 |